2. Specific aims

The discovery of AID has linked the phenomena of SHM and CSR, and gene conversion of the Ig genes and has greatly contributed to the understanding of their underlying mechanisms at the DNA level. Despite the essential role of AID in SHM, CSR and gene conversion, there is the agreement that many crucial factors may remain undiscovered for all three Ig diversifying mechanisms. One aim of this thesis is to document the necessity for additional factors; and to identify candidates for such factors. As a study model we chose the commonly used B cell line WEHI-231 and its derivates WEHI-FS and WEHI-HM.

Specific Aim 1 Study AID expression and the requirement for additional factors in SHM and CSR

WEHI-231 is known as an immature B cell line with no evidence for SHM or CSR. We have found that some WEHI-231 lines express endogenous AID, but nevertheless do not switch the Ig class or hypermutate at a high level. The aim thus was to describe the various WEHI-231 lines with respect to expression of AID and other differentiation markers; and to determine the reason(s) for the differences in AID activity between WEHI-231 and other AID-expressing cells.

Although the early precursor lines of WEHI-231 do not express the canonical 24-kD AID protein, they may express a non-canonical 18-kD form of the AID protein, which could represent a temporal precursor to functional AID. Such a transition in forms may also occur in normal B cell differentiation, and experiments are designed to address this possibility. Although probably not directly related to the AID question, we also wondered why a subclone of WEHI-231 has decreased surface IgM expression, and our aim was to define the underlying lesion(s).

Specific Aim 2 Search for missing factors involved in CSR and SHM

Because WEHI-FS apparently has all the known required factors for CSR and SHM, we aimed to use this line to identify additional factors needed for these two processes to occur. The plan was to use retroviral insertional mutagenesis to either turn on positive factors or destroy negative factors. Furthermore, in a yeast-two hybrid screen, we have identified Supt6h and Tid-1 as AID binding proteins. In this work, we attempted to establish the biological relevance of these findings.